The clinical expression of large and small airway dysfunction in asthma

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CHAPTER 1

General Introduction

GENERAL INTRODUCTION

Asthma is a chronic airway disease affecting approximately 300 million individuals worldwide (1). It is characterized by airway inflammation and bronchial hyperresponsiveness, leading to recurrent episodes with respiratory symptoms. Nowadays, it is widely accepted that asthma affects the total bronchial tree from the large to the small airways. The small airways are usually defined as those with an internal diameter <2 mm and are located from approximately the 8th generation of the bronchial tree (2,3). For many years the exact site of airway inflammation and bronchoconstriction in asthma was controversial. Several decades ago, the large airways were considered as the important site of airway dysfunction in asthma, whereas the contribution of the small airways was thought to be negligible (4). Nowadays, it seems illogical to focus on the large airways only, and in this way disregarding more than 99% of the airways. However, there were several reasons to believe, though incorrectly, that asthma is an isolated large airway disease.

Asthma, a large airways disease?

The paradigm of asthma as a large airway disease found its origins in the 1960s with the new description of the bronchial tree by Weibel (2). In 1915 Rohrer had underestimated the number of branches counting 86 branches with a diameter of 2 mm, and Weibel found 300 to 400 branches with this diameter (5). The findings of Weibel made clear that the number of small airways was much larger, and therefore the resistance of the small airways much lower than previously thought. The cross-sectional area of the bronchial tree exponentially increases towards the end of the bronchial tree, leading to a lower resistance of the small airways compared to the large airways despite the smaller lumen of the small airways (Figure 1). The latter is in line with a study performed in 1967 by Macklem and Mead (6). They measured small airway resistance with a retrograde catheter wedged in the bronchi of dogs and observed that doubling of the small airway resistance would only add 10% to the total airway resistance. Since the contribution of the small airway resistance to the total airway resistance was so small, Mead declared the small airways to be the "lung's quiet zone"(4). This statement was earlier used by Woolcock and colleagues, who supposed that small airway dysfunction would not affect conventional test results and consequently the small airways would remain clinically silent (7). Due to the scarce availability of accurate tests to assess small airway dysfunction, small airway obstruction could only be detected until it became far advanced. In other words, the lack of accurate small airway dysfunction tests has been an important reason to focus predominantly on the large airways.

Until the introduction of the fiberoptic bronchoscope, data on endobronchial sampling of the small airways were limited (8). Biopsies obtained from the large airways provided direct information about airway inflammation and showed an increased eosinophilic inflammation in patients with asthma compared to subjects without asthma (9-11). Investigation of post-mortem tissue of fatal asthma revealed that inflammation was present throughout the bronchial tree including the small airways (12). However, the relevance of these findings was limited as these studies considered only severe life-threatening asthma patients and data was not obtained from in vivo tissue. For a long time, it remained questionable if the inflammatory process involved not only the large conducting airways in asthma but also the smaller airways.

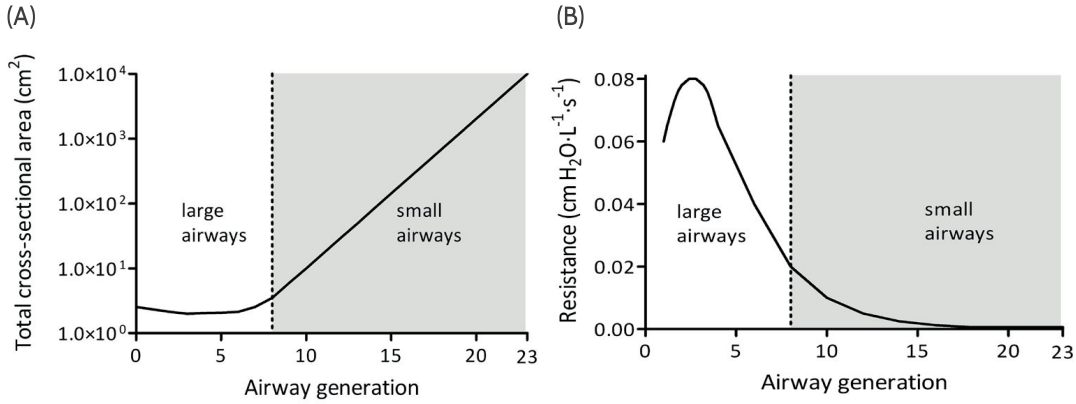

Figure 1. (A) The total cross-sectional area of all the airways in each generation, (B) The total airway resistance of all airways in each generation. This figure is based on the findings of Weibel (2).

Traditionally, asthma has been described as a disease with bronchoconstriction of the large conducting airways, whereas the potency of the small airways to constrict has been doubted for many years (13). This idea was supported by findings of Barnes and colleagues, who showed that muscarinic receptors were abundantly present in the large airways using autoradiographic imaging, whereas muscarinic receptors were nearly absent in the small airways (14,15). They therefore postulated that the greatest bronchoconstriction would occur in the large airways mainly. In addition, Ebina and colleagues investigated post-mortem tissue of asthma patients and showed that smooth muscle hypertrophy was most pronounced in the large airways compared to the small airways (16). This finding supported the idea of Barnes and colleagues that bronchoconstriction would mainly concern the large and not the small airways.

Taken together, because of the minimal contribution of the small airways to the total airway resistance, scarce availability of small airway dysfunction tests, minimal evidence of small airway pathology and the unlikelihood that small airways participate in bronchoconstriction, research of asthma focused predominantly on the large airways and disregarded the role of the small airways (Table 1).

Table 1. Examples of studies supporting the paradigm that asthma was an isolated large airway disease

Asthma, a large airway disease?	
1963 Weibel	Resistance of the small airways is lower than resistance of the large airways
1967 Macklem and Mead	Doubling of small airway resistance adds only 10% to the total airway resistance
1970 Mead	Small airways are lung's "quiet zone"
1983 Barnes *et al*	Muscarinic receptors are nearly absent in the small airways
1990 Ebina *et al*	Smooth muscle hypertrophy of the large airways
1990 Azawwi *et al*	Increased eosinophilic inflammation in bronchial biopsies of asthma patients compared to healthy controls

Small airways in asthma

The interest in the role of the small airways in asthma was renewed with the introduction of new techniques, like the application of peripheral resistance and transbronchial biopsies with the fiberoptic bronchoscopic technique (13). Using fiberoptic bronchoscopy in living humans, Wagner and colleagues observed a sevenfold increase in peripheral airway resistance in patients with mild asthma compared to healthy subjects (17). In line with this, Yanai and colleagues observed that the small airway resistance in patients with asthma contributed to 35-50% of the total airway resistance (18). In a later study by Wagner and colleagues an increased peripheral airway resistance was found in patients with asthma in response to a local challenge of the small airways with histamine (19). Peripheral airway resistance was also shown to be increased in patients with nocturnal asthma compared to patients without nocturnal asthma (20). Overall, these studies suggest that the peripheral airway resistance is increased in patients with asthma and that this increase is related to clinical features such as bronchial hyperresponsiveness and nocturnal asthma.

Pathologic evidence of small airway inflammation has now been provided by studies using transbronchial biopsies (21,22). Kraft and colleagues found an increase in eosinophilic inflammation overnight in the small airways, and not in the large airways, in patients with nocturnal asthma (21). In addition, Wenzel and colleagues observed increased numbers of neutrophils in endobronchial and transbronchial biopsies in patients with severe asthma despite treatment with high dose corticosteroids (22). Extensive research of post-mortem tissue in patients with and without asthma provided additional evidence of small airway inflammation in asthma. Hamid and colleagues investigated the inflammatory process in surgically resected lungs and showed that the number of T-cells, total eosinophils and activated eosinophils was higher in both small and large airway lung tissue of asthma patients compared to controls (23). Additionally, this inflammation was not only present in the inner wall of the airways, between the basement membrane and the smooth muscle, but also in the outer wall, between the smooth muscle layer and the lung parenchyma. Recently, it has been shown by Nihlberg and colleagues and Bergeron and colleagues, using endobronchial and transbronchial biopsies, that the small airways can also be affected by remodeling processes in patients with mild asthma (24,25). Taken together, these pathological studies have shown that inflammatory and remodeling processes affect the total bronchial tree from the large to the small airways.

Nowadays, there is also increasing evidence from numerous clinical and functional studies that the small airways play a role in more severe bronchial hyperresponsiveness. Kaminsky and colleagues performed a local challenge in the small airways with cool dry air using a wedged bronchoscope technique and observed an increased peripheral resistance in patients with asthma after provocation. In line with this, a study of Decramer and colleagues observed an increased peripheral resistance, measured with the FOT, after challenge with cool dry air (26). In addition, Zeidler and colleagues observed increased air trapping in patients with asthma measured with a High Resolution Computed Tomography (HRCT) scan after a cat-room challenge, while there was no fall in FEV_1 (27). These findings suggest that the small airways respond and constrict in response to different environmental stimuli.

The group of Gosens and colleagues has investigated precision cut lung slices of human small airways with videomicroscopy and observed a clear small airway constriction in response to methacholine (unpublished data; personal communication). Application of videomicroscopy and precision cut lung slices enables the direct functional assessment of small airways instead of measuring the presence of muscarnic receptors as performed in previous studies with autoradiographic imaging (14,15). Using videomicroscopy of precision cut lung slices, Brown and colleagues showed that inhibition of muscarinic receptors inhibited smooth muscle contraction of the small airways directly by M3 receptors, and via mediators by M2 receptors in human airways (28). Together, these findings suggest that activation of muscarinic receptors can induce smooth muscle contraction in the small airways.

In summary, recent findings contradict the paradigm that asthma is a large airway disease only and confirm that the inflammatory and remodeling processes in asthma also affect the small airways, and contribute to bronchial hyperresponsiveness (Table 2). The involvement of small airway dysfunction in the clinical expression of asthma is further described in chapter 1.

Table 2. Examples of studies showing that the small airways are importantly involved in asthma

Small airways in asthma!	
1990 Wagner *et al*	A sevenfold increase in small airway resistance in asthma patients compared to healthy controls
1992 Yanai *et al*	Small airway resistance contributes 35-50% of the total airway resistance in patients with asthma
1995 Kaminsky *et al*	Peripheral resistance increases after a local challenge with cool, dry air in patients with asthma
1996 Kraft *et al*	Eosinophilic inflammation increases overnight in transbronchial biopsies of patients with nocturnal asthma
1997 Hamid *et al*	Number of activated eosinophils increases in small airway lung tissue of asthma patients compared to healthy controls
2005 Bergeron *et al*	Airway remodelling is present in the small airways assessed with transbronchial biopsies
2013 Brown *et al*	Cholinergic antagonism of muscarinic receptors in the small airways inhibits smooth muscle contraction
2014 Gosens *et al* (unpublished data)	Small airway smooth muscle cells contract in response to methacholine

Table 3. Tests to assess small airway dysfunction

Test	Parameters of small airways dysfunction	Advantages	Disadvantages
Airway obstruction			
Spirometry	$FEF_{25-75\%,}$ $FEF_{50\%}$	▪ Easy to perform ▪ Low costs ▪ Not time-consuming	▪ Low reproducibility ▪ Influenced by large airway obstruction ▪ Not correlated with inflammation (42)
	FVC/SVC	▪ Good detection of BOS after LTX (43)	▪ Not specific
Resistance			
Impulse oscillometry (IOS)	R5-R20, X5, Fres, AX	▪ Easy to perform ▪ Correlates with $FEF_{50\%}$ ▪ Correlates with MCh-induced changes in ventilation heterogeneity (38)	▪ Difficult to interpret ▪ Relationship with severity of disease not known
Air trapping			
Body plethysmography	FRC, RV, RV/TLC	▪ Non-invasive ▪ Correlates with small airway resistance(20) ▪ FRC correlates with number of eosinophils in transbronchial biopsies (42)	▪ Time-consuming test ▪ Influenced by large airway obstruction
Ventilation heterogeneity			
Single breath nitrogen washout test (SBNT)	CV, CV/VC, slope phase III	▪ Non-invasive ▪ Not time-consuming	▪ Low reproducibility
Multiple breath nitrogen washout test (MBNW)	Sacin, Scond	▪ Very sensitive ▪ Good reproducibility ▪ Correlates with FRC (38)	▪ Not widely available ▪ Time consuming in patients with severe ventilation heterogeneity
Imaging			
High resolution computed tomography (HRCT)	Air trapping	▪ Visual information of air trapping ▪ Related to air trapping measured with body plethysmography (44)	▪ Radiation load ▪ High costs

Table 3. Continued

Magnetic resonance imaging (MRI) with hyperpolarized helium	Regional ventilation defects	▪ More detailed information	▪ Technically demanding ▪ High costs
Inflammation			
Bronchoscopy	Transbronchial biopsy	▪ Direct information of inflammation	▪ Invasive
Sputum induction	Late phase sputum	▪ Non-invasive	▪ Little evidence
Exhaled nitric oxide (eNO)	Alveolar eNO	▪ Non-invasive	▪ Influenced by ICS, smoking (45,46) ▪ Time-consuming test

AX: Reactance area, BOS: bronhiolitis obliterans, CV: Closing volume, $FEF_{25-75\%}$: Forced expiratory flow at 25% to 75% of the FVC, $FEF_{50\%}$: Forced expiratory flow at 50% of the FVC, FRC: Functional residual capacity, Fres: Resonant frequency of reactance, FVC: Forced vital capacity, MRI: Magnetic resonance imaging, HRCT: High resolution computed tomography, IOS: Impulse oscillometry, LTX: lung transplantation, MBNW: Multiple breath nitrogen washout test, eNO: Exhaled nitric oxide, R5-R20: Difference resistance of the respiratory system at 5 Hertz and resistance of the respiratory system at 20 Hertz, RV: Residual volume, Sacin: Ventilation heterogeneity generated in the acinar lung zone, SBNT: Single breath nitrogen test, Scond: Ventilation heterogeneity generated in the conductive lung zone, SVC: slow vital capacity, TLC: Total lung capacity, X5: Reactance of the respiratory system at 5 Hertz

Tests to assess small airway dysfunction

Nowadays, there are several tests available that can measure small airway dysfunction in asthma. Table 3 describes the most frequently used tests with their specific advantages and disadvantages (29,30).

Spirometry is a common test to assess severity of asthma and is able to obtain the forced expiratory flow values, i.e. $FEF_{50\%}$ and $FEF_{25-75\%}$, as variable of small airway function. The $FEF_{25-75\%}$ was shown to be closely related with air trapping on an expiratory computed tomography (CT) scan and with ventilation heterogeneity measured with the multiple breath nitrogen washout (MBNW) test (31,32). A disadvantage of the $FEF_{25-75\%}$ is the low reproducibility compared to the FEV_1.

Resistance measurements of the small airways have received renewed interest to assess small airway dysfunction and are performed with the FOT or impulse oscillometry (IOS). The IOS technique enables measurement of large and small airway resistance (R20 and R5-R20), small airway reactance (X5), and reactance area (AX) reflecting small airway function (33,34). Recently, it has been shown that the IOS technique has a good short-term and long-term reproducibility (35). Boudewijn and colleagues compared symptomatic and asymptomatic asthmatic subjects with bronchial hyperresponsiveness using the IOS (36). They observed that symptomatic subjects with asthma had higher R5-R20 and X5 values, reflecting small airway dysfunction, before and after provocation with methacholine than asymptomatic subjects, while there was no difference in R20, reflecting large airway dysfunction. In line with this, Mansur and colleagues showed that the change in small airway reactance (ΔX5) during the methacholine provocation test was

related to methacholine-induced increase in dyspnea, chest tightness and wheezing (37). The spirometric parameters FEV_1 and $FEF_{50\%}$, reflecting the large and small airway respectively, were only associated with methacholine-induced wheezing, suggesting that IOS is a more sensitive test than spirometry to assess changes in asthma symptoms.

Another technique to assess small airway dysfunction, is the relatively new MBNW technique, which measures ventilation heterogeneity of the small conductive and acinar airways (Scond and Sacin). A study using the MBNW showed that the Scond is related to the FRC, which is a measure of air trapping (38). In addition, a higher Scond was also related to a more severe response to methacholine in asthmatic subjects (39). This study showed no association of the response to methacholine with the Sacin, suggesting that the conductive airways reflect a more important lung zone with respect to air trapping and bronchial hyperresponsiveness than the acinar airways in asthma (39).

The use of imaging techniques to assess small airway dysfunction is a new research field. The HRCT scan is a non-invasive method that cannot measure the small airways directly, but can quantify air trapping as reflection of small airway closure (40). The main disadvantage of HRCT scans is the radiation load. Another imaging technique is the magnetic resonance imaging (MRI) with inhalation of hyperpolarized helium. This technique provides a higher resolution than the HRCT and can visualize regional ventilation defects of the total lung (41). It is a promising technique, however at this time data about associations between ventilation defects and small airway parameters or clinical features are limited. The MRI technique is only available in a few specialized centers.

In summary, there are several techniques available to measure small airway dysfunction assessing different aspects like obstruction, ventilation heterogeneity, air trapping and inflammation. Unfortunately, nowadays there is still no cut-off value or gold standard to define small airway dysfunction.

Small particle treatment

The introduction of the new hydrofluoroalkane (HFA) formulation in the 1990s led to inhalation therapy with small particles of 1-2 µm instead of the conventional inhalation therapy with larger particles of 4-6 µm derived from the chlorofluorocarbon (CFC) formulation. The advantage of smaller particles is the higher total lung deposition and especially a higher small airway deposition (47). While inhalation of large particles (6 µm) achieves a total lung and small airway deposition of 46% and 10% respectively, inhalation of small particles (1.5 µm) achieves a total lung and small airway deposition of 56% and 25% respectively.

Several studies now have investigated the effect of small-particle inhaled corticosteroids (ICS) in asthma and have described an improvement in small airway dysfunction with small-particle ICS. For example, Cohen and colleagues observed significant improvements in alveolar nitric oxide and in methacholine-induced air trapping measured with a CT-scan after a 5-week treatment with

small particle ciclesonide (48). In addition, Thongngarm and colleagues observed significantly higher improvements in ventilation heterogeneity of the small airways, as assessed with the single breath nitrogen washout test, in 30 patients with asthma after 3-month treatment with small-particle HFA-beclomethasone than with the conventional CFC-beclomethasone (49). It is important to mention that HFA and CFC formulations not only differ in particle size, but HFA is also delivered with a softer plume, resulting in a lower oropharyngeal deposition compared to CFC formulation (50,51). Particle size is one of the key factors influencing lung deposition of inhalation medication in the lungs, but also the type of the device, formulation of medication, and inhalation flow are factors that contribute importantly to total lung deposition as well as peripheral deposition. Unfortunately, most studies investigating small particle ICS did not control for all these factors, and we cannot be certain whether the observed effects are due to a difference in particle size. Taken together, small particle ICS seems to improve small airway dysfunction and have clinical benefits, whether this is better than large particles has yet not been proven.

A few studies investigated the effect of different particle sizes of β2-sympathicomimetics on airway obstruction. Weda and colleagues compared salbutamol with a content of 15%, 27% and 67% fine particles (<5.9 μm) and found no difference in the efficacy to improve the FEV_1 (52). In addition, Usmani and colleagues investigated the effect of particles of MMAD 1.5, 3.0 and 6.0 μm albuterol and found that the small particles of 1.5 μm were less efficacious to improve the FEV_1 and $FEF_{25-75\%}$ than both the particles of 3.0 and 6.0 μm (47). It was proposed that this difference can be explained by a difference in dose response curve by a shift from a steep dose-response curve to a flat dose-response curve with smaller particles (53). Unfortunately, the majority of the studies investigating the effect of small particle β2-sympathicomimetica included only spirometry and did not include small airway parameters assessed with IOS or MBNW. Further studies are required including a larger panel of small airway dysfunction tests to determine the effect of different particle sizes of β2-sympathicomimetics on both large and small airway obstruction.

Phenotypes of asthma

Asthma is a heterogeneous disease and so far several phenotypes have been discovered (54,55). Presence of small airways dysfunction has been proposed as a distinct phenotype of asthma with a different clinical expression (56). Haldar and colleagues performed a landmark study using an unbiased cluster analysis to identify new clinical phenotypes (57). Their analysis included several clinical, physiological and inflammatory parameters, i.e. sputum cell counts and exhaled nitric oxide, however parameters of small airway dysfunction were not included. One of the phenotypes identified by Haldar and colleagues was the obese non-eosinophilic asthma patient with increased symptoms. This phenotype is discussed in chapter 8.

Aim of the thesis

The first and main aim of this thesis is to assess whether small airway dysfunction contributes to the clinical expression of asthma. To this end, we reviewed the literature and analyzed different asthma populations investigating the relationship between small airway dysfunction and clinical

features of asthma. Secondly, we aimed to develop new tools that can identify patients with small airway dysfunction. We started with a questionnaire to assess small airway dysfunction and a new provocation test with dry powder adenosine.

Outline of the thesis

Chapter 2 gives a systematic overview of studies investigating small airway dysfunction in relation to asthma control, occurrence of exacerbations, nocturnal asthma, bronchial hyperresponsiveness, exercise-induced asthma and allergen exposure. In addition, we explored the relation between small airway dysfunction and exposure to air pollution and described studies that found an effect of treatment on both small airway function and asthma symptoms.

Chapters 3 and 4 investigate the association between small airway dysfunction and specific clinical features of asthma. Chapter 3 focuses on the role of small airway dysfunction in asthma symptoms and bronchial hyperresponsiveness in a study population of 58 patients with mild to moderate-severe asthma, who were extensively characterized with measurements of lung function, impulse oscillometry, exhaled nitric oxide and a methacholine provocation test. Chapter 4 is an observational study in 3,155 asthma patients derived from primary care focussing on the association of small and large airway function with control of asthma, and the response to specific environmental stimuli.

Chapter 5 describes the first step in the development of a small airway dysfunction questionnaire. A new small airway dysfunction tool may help to identify asthma patients with small airway dysfunction. In order to select relevant differences in signs and respiratory symptoms between asthma patients with and without small airway dysfunction, both groups of asthma patients are asked about their perceived asthma symptoms in individual in-depth interviews and in focus groups

Chapters 6 and 7 introduce a new provocation test with dry powder adenosine. The proof of principle in five asthma patients is presented in chapter 6. In chapter 7 we try to challenge the small and large airways selectively with small- and large-particle dry powder adenosine and inhaled with either a low or high flow rate. We hypothesize that a small-particle slow-inhalation provocation test gives a higher small airway deposition and thus a higher response in the small airways than a test with large particles and/or inhalation with a high flow rate. Based on a differential response to the four adenosine challenge tests, we try to identify patients with small airway dysfunction.

Chapter 8 analyzes eosinophils in sputum and bronchial biopsies in obese and nonobese subjects with mild-to-moderate asthma. This study is performed in response to an article of Desai and colleagues showing that eosinophils in biopsies were elevated in obese patients compared to nonobese patients with severe asthma.

Chapter 9 summarises and discusses the results of all articles and gives my future perspectives.

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